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A Study of In-Vitro Hypoglycemic and Glucose Uptake Activity Ethanolic Extract of Syzygium Cumini Linn

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ABSTRACT

Natural goods, which come from living things, are very important in pharmacognosy, medicine, and agriculture. This study explores the anti-inflammatory actions and phytochemical characteristics of *Syzygium cumini*, also known as black jamun, a plant with therapeutic potential for the treatment of diabetes and related inflammatory disorders. Using Fourier Transform Infrared Spectroscopy (FTIR) and High-Performance Thin-Layer Chromatography (HPTLC) to characterize *Syzygium cumini* chemically and validate the presence of important bioactive components like Hydroxy Citric Acid (HCA), the research focuses on this process. An anti-inflammatory model generated by diabetes was created to assess the effectiveness of the *Syzygium cumini* extract levels and inflammatory markers such as TNF-alpha and IL-6. Furthermore,3T3-L1 adipocyte cells were used in in vitro glucose uptake tests. The outcomes demonstrated that, at lower concentrations, *Syzygium cumini* improves glucose absorption; peak effectiveness was noted at 20 µg/ml. This shows that *Syzygium cumini* may be useful in controlling hyperglycemia and inflammation in diabetes due to its insulin-sensitizing qualities. Higher extract concentrations, however, decreased glucose absorption, suggesting a potential cytotoxic effect. Overall, the research highlights *Syzygium cumini* promise as a natural medicinal agent for the treatment of diabetes. The results indicate that more investigation into the bioactive substances and modes of action of *Syzygium cumini* is necessary in order to potentially create novel therapies for diabetes and its sequelae.

Keywords: *Anti-inflammatory model, Cell Line Study, Diabetes Mellitus, FTIR, Glucose Uptake Assay, HPTLC, In Vivo activity, Phytochemistry, Syzygium cumini Linn*

1. INTRODUCTION

Natural products are chemical compounds or substances derived from living organisms. The chemistry of natural products encompasses their structure, physical and chemical characteristics, biosynthesis, extraction, identification, quantification, and reactivity. These compounds are produced through primary or secondary metabolic pathways. Metabolism refers to a sequence of enzyme-catalyzed biochemical reactions occurring within an organism's cells, which are essential for its growth, development, and adaptation to environmental conditions. Metabolism is categorized into two types: anabolism and catabolism. The term "metabolites" generally refers to small molecules that serve as intermediates or end products of metabolism. Primary metabolites are crucial for optimal development, growth, and reproduction; examples include proteins, fats, carbohydrates, and alcohol.

Natural products represent a broad and heterogeneous class of chemical substances with diverse biological activities. These compounds have been widely utilized across various fields, including agriculture, veterinary medicine, and human medicine. They are derived from bacterial, fungal, plant, and marine animal sources(1-4)

Role of Plant-Based Natural Products in Medicine

Plant-based natural products play a significant role in medicinal applications due to their antimicrobial, antibacterial, and therapeutic properties. The study of bioactive natural products, known as pharmacognosy, provides the necessary tools for the identification, selection, and preparation of medicinally valuable compounds. In natural extracts, organisms typically

produce a single active compound or a group of related molecules with biological activity. These bioactive molecules can be directly used in drug discovery and development, either in their natural form or through structural modifications to enhance efficacy or reduce adverse effects (5)

Phytochemistry is a branch of chemistry that focuses on plant-derived compounds, particularly secondary metabolites. This discipline explores the structure, biosynthesis, and functions of these metabolites. Plants produce phytochemicals for various reasons, including defense against pests and diseases. Major classes of plant metabolites include terpenes, glycosides, polyphenols, and alkaloids. A phytochemical investigation of plant materials typically involves species authentication, followed by extraction, separation, isolation, structural elucidation using spectroscopic and chromatographic techniques, and biological evaluation (6).

Traditional medicinal systems such as Ayurveda (India), Unani (Greece), Traditional Chinese Medicine (China), and the Amachi system (Tibet) were once regionally restricted but are now widely recognized globally. The World Health Organization (WHO) has acknowledged the scientific potential of traditional medicine and actively promotes research into its efficacy. Consequently, phytochemical methods play a crucial role in screening and analyzing bioactive compounds for therapeutic mechanisms and quality control (7).

Natural product-based approaches to diabetes management involve the use of herbs, plants, minerals, and other bioactive substances to regulate blood sugar levels and improve insulin sensitivity. Common natural agents used for diabetes management include apple cider vinegar, chromium, magnesium, alpha-lipoic acid, fenugreek, cinnamon, berberine, bitter melon, and Gymnema sylvestre. These natural remedies should complement conventional treatments, and it is essential to consult a healthcare professional before initiating any new regimen.

Diabetes mellitus (DM) is characterized by chronic hyperglycemia, defined as high blood glucose levels either during fasting or after meals. Prolonged hyperglycemia is associated with damage, dysfunction, and potential failure of various organs and tissues, including the kidneys, retina, heart, nervous system, and blood vessels. These complications significantly impact health and reduce quality of life. According to the International Diabetes Federation (IDF), the global prevalence of diabetes is projected to rise from 366 million in 2011 to 552 million by 2030. Diagnostic criteria established by the World Health Organization (WHO), the Canadian Diabetes Association (CDA), and the American Diabetes Association (ADA) define diabetes as: A fasting plasma glucose level of ≥126 mg/dL (7.0 mmol/L) on two or more occasions. A random plasma glucose level of ≥200 mg/dL (11.1 mmol/L). A plasma glucose level of ≥200 mg/dL (11.1 mmol/L) two hours after a 75 g oral glucose tolerance test (OGTT)

There are three primary types of diabetes:

1. Type 1 Diabetes: Previously referred to as "juvenile diabetes" or insulin-dependent diabetes mellitus (IDDM), this type results from an autoimmune attack on insulin-producing pancreatic β-cells.
2. Type 2 Diabetes: Formerly called "adult-onset diabetes" or non-insulin-dependent diabetes mellitus (NIDDM), it is primarily associated with insulin resistance, often linked to obesity and a sedentary lifestyle.
3. Gestational Diabetes: A temporary form of diabetes occurring during pregnancy in women who have never had diabetes before.

Prevention and management strategies for diabetes include a balanced diet, regular physical activity, smoking cessation, weight management, blood pressure control, and proper foot care.

Syzygium cumini, commonly known as black jamun, Java plum, Indian blackberry, or jambolan, is a significant medicinal plant belonging to the Myrtaceae family [5]. Native to India and Indonesia, this large evergreen tree thrives in diverse agroclimatic zones of South Asia. It is widely distributed but remains underutilized despite its extensive medicinal properties [6]. The tree can grow up to 30 meters in height and has a lifespan exceeding 100 years. Phytochemical studies of ethanol extracts from jamun stem bark, leaves, seeds, and fruit pulp have revealed the presence of alkaloids, anthraquinone glycosides, flavonoids, tannins, saponins, phenols, cardiac glycosides, terpenoids, phytosterols, steroids, and amino acids [7], [8]. Notably, terpenoids and phytosterols are absent in leaf extracts. Jamun seeds contain high levels of protein and calcium, along with bioactive compounds such as gallic acid (1-2%), ellagic acid (19%), and tannins (8-9)

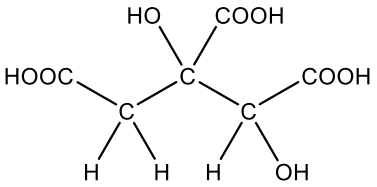
Diabetes is often associated with chronic inflammation, characterized by elevated levels of inflammatory cytokines such as interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α). These cytokines contribute to insulin resistance and exacerbate diabetic complications. Syzygium cumini has demonstrated anti-inflammatory, antioxidant, and anti-diabetic properties due to its tannins, flavonoids, alkaloids, and essential oils. Experimental studies will involve multiple groups:

1. Non-diabetic control group (no treatment)
2. Diabetic control group (untreated diabetes)

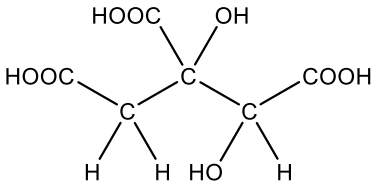
3. Diabetic + Syzygium cumini extract group (receiving plant extract)

Hydroxycitric acid (HCA), a bioactive compound in Syzygium cumini, exists in two stereoisomeric forms: (+) HCA and (-) HCA. Chemical characterization was performed using High-Performance Thin-Layer Chromatography (HPTLC) and Fourier Transform Infrared (FTIR) Spectroscopy. The presence of HCA was confirmed through spectral analysis, exhibiting characteristic peaks in the 1600–1070 cm^{-1} range. The extracted bioactive compound showed a positive peak in HCA analysis, highlighting its potential role in glucose uptake (10).

This study aims to explore the therapeutic potential of Syzygium cumini in diabetes management, emphasizing its anti-inflammatory and bioactive properties.



(+)-Hydroxycitric acid



(-)-Hydroxycitric acid

2. MATERIAL AND METHODS

Extraction

To generate calcium hydroxy citrate salt, mix 150 grams of dried *Syzygium cumini* with 750 millilitres of distilled water using a magnetic stirrer. This should not take more than 30 minutes. Use Whatman filter paper No. 1 to filter the mixture in order to collect the filtrate. This filtrate should be mixed with 1 N calcium hydroxide solution after the pH reaches 7.0. To remove the calcium salt and induce the precipitation of hydroxy citrate (HCA), filter the mixture. Finally, dry the collected calcium hydroxy citrate salt in a hot air oven [14], [15].

3T3-L1 cell differentiation into adipocytes

To culture and differentiate 3T3-L1 cells for glucose uptake tests, we seeded 5×10^3 cells/well in 96-well plates at passage 9. We utilized DMEM/F12 medium with 10% FBS. When we transferred the media to differentiation medium (DMEM/F12 + 2% FBS, 10 g/mL insulin, 0.5 mM IBMX, and 1.0 mM dexamethasone) two days after confluence, a rounded phenotype was developed. For a total of four days, the culture medium for the cells was replaced every two days. Then, in place of the differentiation medium, DMEM/F12 + 2% FBS was used for the glucose absorption experiment (11-12)

Uptake assay glucose

On the assay day, 3T3-Adipocyte cells were treated in triplicate with varying doses of the test chemicals (10 g/mL, 20 g/mL, 40 g/mL, and 80 g/mL) and left without insulin for five hours. Following the incubation period, the leftover liquid was disposed of, and the amount of glucose in the remaining mixture was determined by measuring the absorbance at 570 nm using the DNSA technique. A zero control was used to compare the glucose levels. Using the MTT assay, the test substances' percent viability was calculated using the following formula:

Percent Increase=c/t×100

where t is the optical density measurement of the test substance, and c is the optical density of the vehicle control.(16).

3. RESULTS

Interpretation of HPTLC results:

The results of the HPTLC 3D plot (Figure 1) illustrate the absorbance of different compounds across the plate at the measured wavelength. The horizontal axis represents the position on the plate, the y-axis corresponds to the wavelength, and the vertical z-axis denotes the absorbance intensity.

For Track 1, corresponding to Hydroxy Citric Acid, the peak analysis revealed a start position at 0.36 Rf, a maximum height position at 0.54 Rf, and an end position at 0.62 Rf. The peak exhibited a height of 1164.0 AU and an area of 5989.3 AU. Additionally, an unidentified peak was observed, indicating the presence of another compound in the sample that has not yet been identified.

The HPTLC analysis effectively identified and quantified Hydroxy Citric Acid in the sample. The significant peak area and height suggest a notable concentration of the compound. However, the presence of an unknown peak implies that an additional compound may be present, necessitating further investigation to determine its identity.

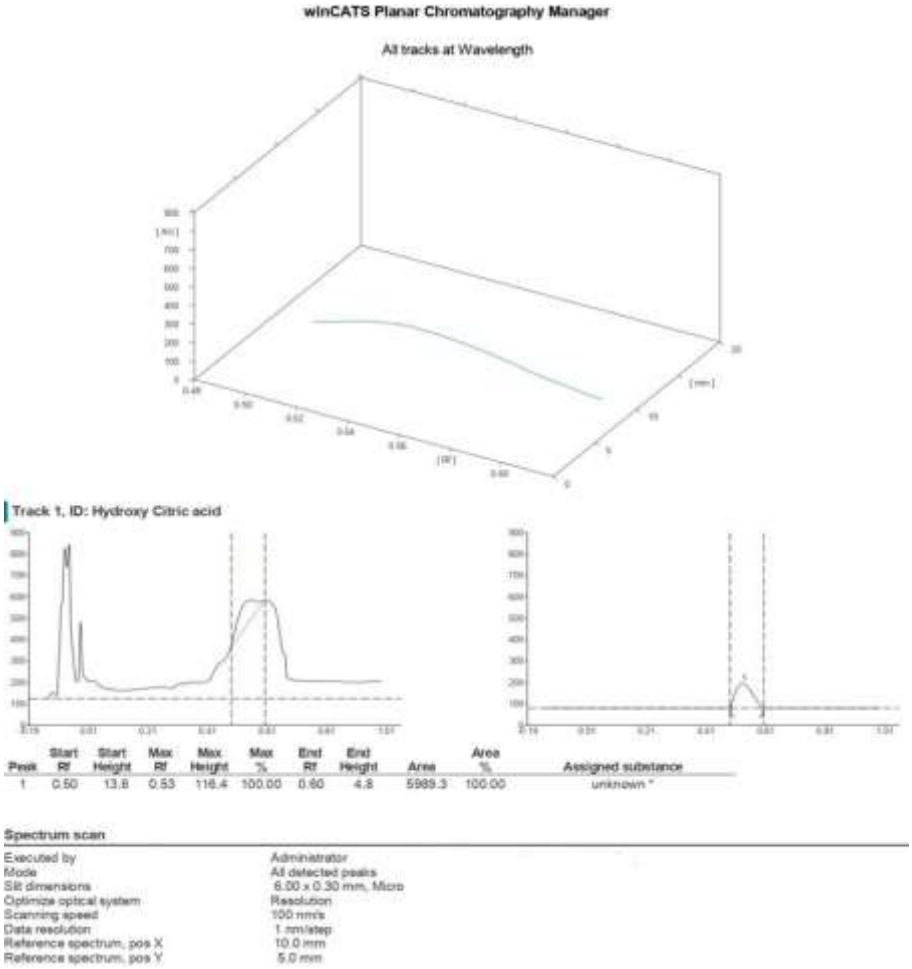


Figure 1: HPTLC Spectrum of hydroxy citric acid.

Interpretation of FTIR Spectrum: The FTIR spectrum (Figure 2) presents the transmittance (%) against wavenumber (cm^{-1}), highlighting key absorption bands corresponding to different functional groups in the sample. The broad O-H stretching vibration at 3424.63 cm^{-1} is characteristic of hydroxyl groups found in alcohols and phenols. The C-H stretching vibrations observed at 2929.55 cm^{-1} and 2870.79 cm^{-1} indicate the presence of aliphatic methylene groups. A peak at 2141.83 cm^{-1} suggests a possible C≡C stretching vibration, which is typical of alkynes. The strong C=O stretching vibration at 1683.94 cm^{-1} signifies the presence of carbonyl-containing functional groups such as ketones, aldehydes, or carboxylic acids.

Further analysis of the spectrum reveals C=C stretching vibrations at 1599.38 cm^{-1}, 1519.70 cm^{-1}, and 1452.02 cm^{-1}, indicative of aromatic rings. The C-H bending vibration at 1379.44 cm^{-1} points to the presence of methyl groups. The characteristic C-O stretching vibrations at 1228.60 cm^{-1} and 1136.68 cm^{-1} suggest the presence of alcohols, ethers, or carboxylic acids, while the C-N stretching vibrations at 1038.21 cm^{-1} and 1006.44 cm^{-1} indicate amines or amides. Out-of-plane bending vibrations at 888.57 cm^{-1}, 824.22 cm^{-1}, and 720.20 cm^{-1} suggest the presence of substituted aromatic compounds. Additionally, a possible C-Br stretching vibration at 600.76 cm^{-1} indicates the presence of organobromine compounds.

Overall, the FTIR spectrum provides a detailed molecular fingerprint of the sample, confirming the presence of hydroxyl, aliphatic, carbonyl, aromatic, ether, amine, and possible organobromine functional groups. The presence of these diverse functional groups suggests a complex molecular composition that warrants further structural analysis.

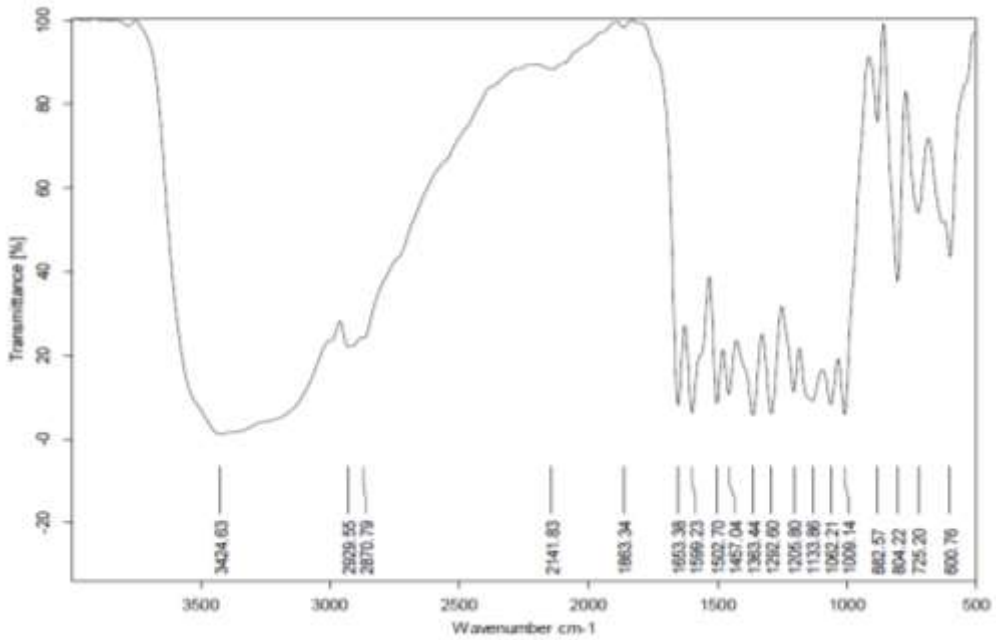


Figure 02: FTIR spectrum of hydroxy citric acid

Evaluation of Glucose uptake activity of *Syzygium cumini Linn*

The (Table:1) presents data on the percentage of glucose absorbed by 3T3-L1 cells in the presence and absence of insulin at varying test sample concentrations. The table includes a serial number for each test concentration, specifying the corresponding concentration of the test samples in micrograms per milliliter (µg/ml) used in the assay. The tested concentrations are 10, 20, 40, and 80 µg/ml, providing insights into the glucose uptake efficiency under different conditions.

Table1: Assessment of antidiabetic properties of test samples using glucose uptake assay on 3T3-L1 cells

Sl.No.	Concentration(µg/ml)	Percentage of Glucose Absorbed	
		Insulin (Present)	Insulin (Absent)
1	10	85.71429	138.2022
2	20	91.83673	158.427
3	40	77.55102	137.6404
4	80	61.22449	116.2921

The percentage of glucose absorbed by 3T3-L1 cells was evaluated under conditions with and without insulin at varying test sample concentrations. When insulin was present, the glucose absorption percentages were 85.71%, 91.84%, 77.55%, and 61.22% for concentrations of 10, 20, 40, and 80 µg/ml, respectively. In the absence of insulin, the recorded percentages were 138.20%, 158.43%, 137.64%, and 116.29% for the same respective concentrations.

A decreasing trend in glucose uptake was observed with increasing concentrations of the test samples from 10 to 80 µg/ml, suggesting a possible inhibitory effect on glucose absorption by the cells when insulin was present. A similar trend was noted in the absence of insulin, although the overall glucose uptake was higher compared to the insulin-present condition. This indicates that the test samples might exhibit an insulin-mimetic or insulin-sensitizing effect.

The findings suggest that the test samples may influence glucose metabolism and could have potential antidiabetic properties by modulating glucose uptake in cells.

The graph (Figure 03) represents the percentage of glucose uptake at varying concentrations of the test substance. At a concentration of 10 µg/ml, glucose uptake was recorded at 138.20%, indicating a moderate increase from the baseline. The uptake peaked at 158.43% at 20 µg/ml, suggesting maximum efficacy of the test substance at this concentration. However,

at 40 µg/ml, glucose uptake declined to 137.64%, and at 80 µg/ml, it further decreased to 116.29%, demonstrating a significant reduction at higher concentrations.

These findings indicate that the test substance enhances glucose uptake at lower concentrations, with the highest effect observed at 20 µg/ml. Beyond this concentration, a decline in glucose uptake is evident, which may be attributed to a threshold effect or potential cytotoxicity at elevated concentrations. The decrease in glucose uptake at 40 and 80 µg/ml could result from factors such as feedback inhibition, saturation of glucose transporters, or an adverse impact on cell viability.

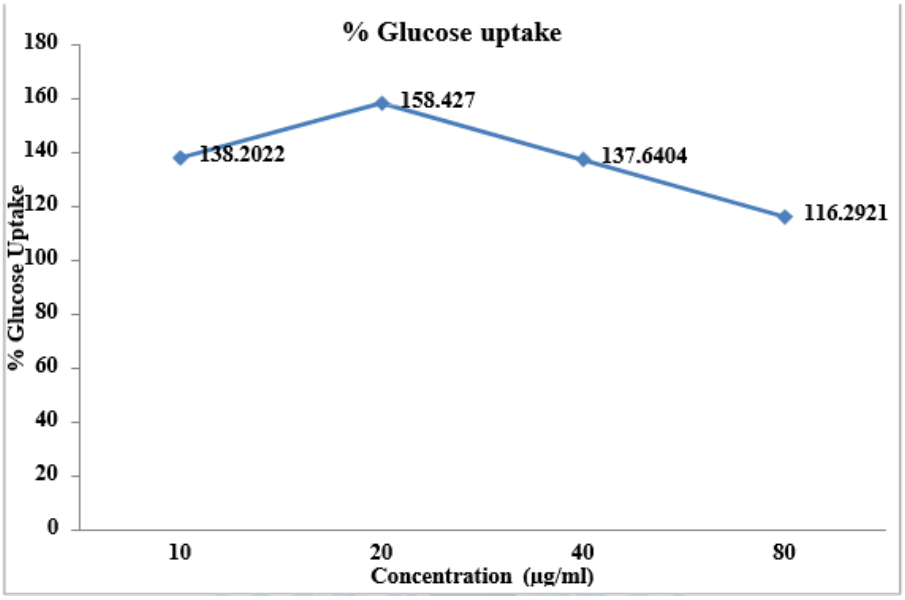


Figure 03: Glucose uptake assay used to test the antidiabetic potential of insulin samples on 3T3-L1 cells

4. CONCLUSION

The study successfully extracted calcium hydroxy citrate salt from Syzygium cumini and characterized its composition using HPTLC and FTIR analyses. HPTLC confirmed the presence of Hydroxy Citric Acid with a significant peak, while FTIR analysis revealed a diverse range of functional groups, including hydroxyl, carbonyl, aromatic, and possible organobromine compounds, indicating a complex molecular structure.

The glucose uptake assay demonstrated that the test samples influenced glucose metabolism in 3T3-L1 cells, exhibiting a concentration-dependent effect. In the presence of insulin, glucose uptake showed an initial increase followed by a decline at higher concentrations, suggesting potential inhibitory effects. In the absence of insulin, glucose uptake was generally higher, indicating possible insulin-mimetic or insulin-sensitizing properties. The highest glucose uptake was observed at 20 µg/ml, beyond which a decline suggested a threshold effect or potential cytotoxicity.

These findings highlight the potential antidiabetic properties of Syzygium cumini-derived compounds. The observed effects on glucose uptake warrant further investigation into their mechanism of action, potential therapeutic applications, and safety profile for diabetes management.

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